BLOOD CHEMICAL CHANGES IN EXPERIMENTAL SETARIA CERVI INFECTION

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This is to certify that the dissertation for M.Phil. course in Zoology has been completed by Miss Humaira Khatoon under my supervision. It is original in nature and I have permitted the candidate to submit it for the award of M. Phil degree in partial fulfilment of the Ph.D. requirements in Zoology.

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(Jamil A. Ansari)

CONTENTS

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I. INTRODUCTION

In spite of spectacular advances in our struggle against parasite, it is obvious that the battle is far from won. There is every chance of parasitic infection. When a susceptible host is infected, profound changes occur in its organs and system. The changes reflect the specific characteristics of a particular infection. As might be expected, the effects are very varied and in many cases represent a combination of several entitis. Biochemical composition of a particular tissue or fluid of body is definite. Any change from this definite composition leads to 'abnormality' which in severe cases give rise to chronic diseases. Several investigators have pointed out that the destruction of the tissue and irritation caused by microfilariae might result in the production of toxic materials and chemical changes in the blood. Although extensive biochemical work on the tissues and blood infected with parasites has been done. But comparatively little experimental work on filarial infections could be carried out because of their strict host specificity.

Recently some reports of accidental infection of *Setaria cervi*, a filarial worm of cattle came to knowledge. The

parasite could easily be implanted in experimental laboratory animals such as white rats, rabbits, guinea pigs etc. This gave an excellent opportunity to make investigations on this aspect of host parasite relationship in labs. Setaria cervi has a wide distribution and heavy incidence of the parasite occurs specially among cattles. It has great economic value because of its association with "lumbar paralysis" and "cerebrospinal nematodiasis" in other animals including man. A series of experiments were planned using white rats as alternate hosts for this worm. Biochemical examination of blood was carried out in detail and changes in different constituents were compared with the normal values. It is very much hoped that the information thus obtained would help in the treatment of the diseased cattle and its consequent eradication.

II. LITERATURE REVIEWED

Very little experimental work has been done on biochemical aspects of the blood of infected hosts and particularly no substantial study has been carried out against *Setaria cervi* infection.

Hartman *et al.* (1939) investigated the blood of 3 dogs highly infected with *Trichinella spiralis*. Calcium, phosphorus, nonprotein nitrogen and chlorine were measured as well as observations on food intake for about 6 weeks following infection was recorded. No marked change in various constituents was observed except for the latter which fell off during the first week and latter increased gradually. Loss of apetite was thought to be the primary result of *Trichinella* infection, and the changes in blood chemistry was due to lower food consumption.

Hamann (1943) studied the changes of histamine in blood and other tissues of rats and guinea pigs infected with *Trichinella spiralis*. The experiment indicated an increase of histamine in the blood and possibly in certain other tissues of the infected animals. Although the formation of this substance might be expected as a result of the irritation and destruction of tissue by migrating larvae, the possibility may also be that

the presence of the worms and their metabolites would produce an anaphylactoid condition with concomittant increase in histamine.

Bullock (1953) observed the appearance of strong alkaline phosphatase activity in the muscle fibres after 4-5 days of infection with Trichinella spiralis larvae.

Wilson (1961) studied the serum protein changes in lambs and kids after exposure to the thread lungworm Dictyocoulus filaria. These animals were infected with 2,500 or 5,000 infective larvae and were examined at weekly intervals for changes in their serum proteins. With the growth of the worms the percentage of globulin showed a gradual increase. When the initial infection became well established the ratio of albumin to globulin decreased. These changes were approximately proportional to the worm burden. The serum proteins returned to within the normal range about 8 weeks after larval production ceased. Neither initial nor challenge infection elicited any change in the serum proteins of those animals which appeared to be naturally resistant to lungworms.

Paul Berghen (1966) studied serum protein changes in Capillaria obsignata infection in two species of birds. Regardless of the number of mature parasite these animals showed remarkable blood protein fluctuations. The rise in total protein and total globulin due to the increase of β - and γ - globulin was

statistically significant. Infected chicks showed elevated total protein levels. While albumin fractions were relatively constant, total globulin was considerably higher than in control animals. This hyperglobulinemia could be accounted for by an increase in β - and γ - globulins which may be associated with resistance of chickens to this parasite and also in continuation of any serious infection.

Haematological values were worked out by Dobson (1967) in worm free and experimentally infected sheep with Oesophagostomum columbianum. Lambs in groups (13-15 months of age) were infected with the larvae, and a group of wormfree lambs were used as controls. Blood samples were taken weekly and packed cell volume, haemoglobin concentration, red cell, total leucocyte, eosinophil leucocyte and differential cell counts were estimated. The sheep showed loss of red cell and haemoglobin and diminished pack cell volume. The values decreased with the severity of the infection dose. A general increase in white cell counts was observed throughout the infection.

Synder et al. (1967) worked on the blood chemical and cellular changes in canine dirofilariasis. They reported an increase in glucose, SGO-T and SGP-T, globulin and decrease in albumin, calcium, sodium, potassium and phosphorus.

Bremner (1969a) studied the values of plasma iron, iron binding capacity and reticulocyte responses in blood of calves

infected with Oesophagostomum radiatum. Worm free calves were paired fed with experimentally infected calves. About 200-500 ml of blood drawn out daily to produce anaemic condition. Similar to that which developed in their infected partners. A third group of calves remained untreated. Plasma iron binding capacity fell below normal in both bled and parasitized calves, but later rose to levels greater than those of untreated calves. Infected calves displayed lower serum protein levels and less efficient utilization of feed than the bled animals. The results indicated that the anaemia of oesophagostomiasis was due primarily to blood loss.

Ishizaki (1970) investigated the hookworm infection, Necator americanus, from the clinical angle by conducting a survey in a village near Tokyo, Japan. The critaria for investigations were haemoglobin contents, bleeding times, red cells, reticulocyte and eosinophil counts, coagulation time, serum iron contents, liver function test, skin test using N. americanus antigen. The level of anaemia showed wide variations, depending on worm load, iron deficiency and other factors. Serum γ - globulin increased in population to the intensity of infection and also to the reticulocyte count. Symptoms were found to be of two kinds, one related to the intensity of infection and the other to the level of anaemia. Some healthy infected persons showed no anaemia.

Laclaelli et al. (1970) worked on the heptoglobin and serum creatin phosphokinase activity in experimentally infected rabbits with Trichinella spiralis. The heptoglobin levels in serum of 3 rabbits did not change. Whereas creatine phosphokinase levels showed a marked increase between the 20th and 55th day of infection.

Saraya et al. (1970) made a study of iron and protein deficiency in hookworm infestation. They observed anaemia in 80% of the patients which was due to loss of blood. Loss of iron and protein due to blood loss lead to anaemia.

Lablonowska (1971) studied the changes in the level of acid soluble phosphate compounds in blood and liver of guinae pigs during experimental infection with Ascaris. The levels were lowered and dependent on the intensity of infection and the duration of infection. Most of the changes occurred during the initial acute larval stage when a lethal dose was diminished, the changes occurred earlier and were significant.

Singh et al. (1971) worked on serum proteins and lipid changes in ancylostomiasis before and after treatment. Serum proteins and lipids were estimated from four groups of persons. The first group comprised of 20 normal subjects, second group of 15 patients with hookworm anaemia, third group of 15 patients positive with hookworm infection but without anaemia and 15 patients with iron deficiency due to chronic blood loss were

categorised as group IV. Serum proteins and lipids were found to be lower in the 3 groups of patients as compared with the normal subjects, the difference being greatest in the patients with hookworm anaemia. Treatment of anaemia with parenteral iron in group II and IV resulted in serum protein and lipid approaching to normal levels in group IV and a partial improvement in group II. Removal of worm with an anthelmintic in patients in group II and III produced significant changes in proteins and lipids indicating that the parasite itself as well as the anaemia, played a part in altering these fractions.

Tantengco et al. (1971) studied the serum and red cell folate activity in Philipino school children with hookworm anaemia. In Philipines 55 of 146 hookworm infected children had haemoglobin level ranging from 4.4 to 11.6%, of these anaemic children, the majority showed microcytosis, hypochromia and anisocytosis in their blood smears and 15 (27.7%) children had serum in their blood folate levels below 3 mμg/ml, of all the infected subject. Serum and red cell folate levels suggestive of folic acid deficiency occurring 30.7 and 40.4% respectively.

Singh et al. (1972-73) studied the blood chemical changes and chemotherapy of buffaloes infected with Setaria cervi and Onchocerca armilata displayed circling movements, reduced milk yields, swellings of the legs and raised body temperature. Increased ESR, SGO-T and SGP-T, serum globulin and glucose levels

and decreased serum albumin, calcium, phosphorus, sodium and potassium and low haemoglobin levels were observed. Serum creatinine remained unchanged and total serum proteins showed little increase. Peripheral microfilaremia and clinical symptoms disappeared after 4 intramuscular injections of 15 ml lithium antimony thiamalate, each at 48 hours intervals.

Klunklin (1972) worked on the serum transminase and hepatic pathology in Trichinosis. Serum glutamic oxalacetic transminase and serum glutamic pyruvic transminase levels were found to be raised respectively in 21 and 25 from a total of 42 patients when examined in 3rd and 5th week of infection. The degree of SGO-T and SGP-T elevation in the group treated with thiabendazole was not statistically different from that in the untreated group.

Boron et al. (1972) worked on the changes in proteins and leucocytes during trichinosis. Blood changes showing decreased albumin 52.35% increased α-2-globulin (10.22%) and γ-globulin were recorded during an 8 week period of observation in 147 patients. The leucocyte count increased in 2nd week to 11,244 mm^3 and there was also a tendency towards a low neutrophil count by the 7th week. The lymphocyte count (30% approximately in the Ist week) decreased from the 3rd to 7th week.

Roychoudhry (1973) demonstrated the electrophoretic pattern of serum proteins in filarial patients. The serum proteins of 86 chronic filarial carriers, 10 acute cases with lymphangitis, lymphadanitis, 17 carriers of W. bancrofti (average of 21.6 micro/20 mm^3 of blood) and 6 normal controls were studied. The chronic cases and carriers had significantly lower mean albumin/globulin and albumin/ γ -globulin ratios than the controls. Acute cases and carriers had significantly lower albumin and β -globulin ratios. The results appear to suggest an increase in the globulin level in infection. The above ratios appear to be lowest in those cases with 35 to 44 micro/20 mm^3 blood.

Samsom et al. (1974) studied the changes in the concentrations of serum urea nitrogen, albumin globulin, sodium and inorganic phosphorus in weaner pigs infected with Trichuris suis. Changes in the blood chemistry of weaner pigs experimentally infected with 15,000 to 100,000 Trichuris suis ova were related to the severity of the clinical signs of the disease. Infection with 15,000 ova produced no clinical and significant biochemical changes. Infection with 50,000 ova caused progressively small decrease in inorganic phosphorus concentration, marked decrease in serum sodium concentration, increase in urea concentration, fall in serum albumin concentrations and rise in globulin concentration. Infection with 100,000 ova caused more rapid fall in serum sodium concentration and increase in serum globulin.

III. MATERIALS AND METHODS

(General)

Albino rats used in these experiments were bred in laboratory and care was taken to prevent all possible external infections. All the rats utilised in the experiments were of the same age group and weight. Adult *S. cervi* worms were collected from freshly slaughtered cattles and stored in physiological saline maintained at 37°C. Transplant of the worm was made within 2 hours of collection. A fine incision of about 2 cm was made in the abdomen of the anaesthetized rats and worms (3 ♀s and 2 ♂s) were slipped into the body cavity. The opening was sutured and murchurochrome was applied to prevent from external infections. The rats inoculated in this way continued to be healthy, and no internal treatment was given to them.

The rats were checked after 7-8 days for microfilariae and for this purpose blood was taken out from the tail. One drop of blood was taken on the slide and one or two drops of distilled water was added for haemolysis and slide was observed under high power of microscope, to check the microfilariae. Presence of microfilariae in the blood showed that the infection

was positive. The rats in group of 4 or 5 were kept in different cages. Weekly sample of blood was drawn with the help of syringe and biochemical analysis was carried out.

IV. INVESTIGATION

1. Protein

(a) Total serum protein:

Experimental procedure.

Quantitative determination of protein was done in normal and infected rats by Lawry Method.

Reagents- (1) 2% sodium carbonate in 0.1 N sodium hydroxide (A)

(2) 0.5% copper sulphate in 1% sodium potassium tartrate (B)

(3) Mixed 50 ml of Reagent A with 1 ml of Reagent B - This is Reagent C.

(4) Folin Phenol Reagent

0.5 ml of serum was taken. Protein was precipitated by 3 ml of TCA. Centrifuged and aliquot was taken for analysis. The aliquot was washed with 5 ml of 0.1 N potassium acetate prepared in absolute alcohol. Centrifugation and washing was repeated with potassium acetate again. The aliquot was then washed and centrifuged with absolute alcohol. The same process was repeated with solvent ether. In all cases washing and centrifugation was done twice with 5 ml portion of solvents. The precipitate was dissolved in 5 ml of 0.1 N sodium hydroxide. 1 ml of aliquot was taken and estimated the protein as follows.

Reagents	Blank (ml)	Samples in ml			
		1	2	3	4
Aliquot	-	0.1	0.1	0.1	0.1
Water	1	0.9	0.9	0.9	0.9
Reagent C	5	5	5	5	5
Folin Phenol Reagent	0.5	0.5	0.5	0.5	0.5

After 10 minutes the colour was read in photometer at 540 mμ (standard curve was plotted earlier before this using serum albumin).

Results and discussion:

Total serum protein was estimated quantitatively both in normal and infected rats (Table 1). Result (Fig. 1) shows little increase in serum total protein. In the first week there is almost no change but afterwards the level ranges from normal value 5.32 gm/100 ml to 6.08 gm/100 ml. The data shows that increase is due to parasitic infestation which induces an increase of gamma globulin. Berghen (1966) studies on the serum protein changes in Capillaria obsignata infections in chickens also presented no appreciable change in the amount of total protein in the Ist and 2nd week of infection. It showed progressive changes from the fourth week onward and that the increase of total protein was definitely due to an important

rise of β - and γ - globulins. It is obvious that changes in blood serum could only be observed during the presence of mature parasites. This could possibly point to lack of permanent immunity. Synder et al. (1967) observations on the blood chemical and cellular changes in 'canine dirofilariasis' showed an increase in total serum protein level in infected dogs than in normal dogs. His finding is in full agreement with the present study. Whereas, Bremner (1969) reports on experimental bovine oesophagostomiasis indicated decrease in serum protein concentration. The values first fell within the normal range 5.8-7.0 gm/100 ml upto the third week and decreased until a minimum mean value of 3.7 gm/100 ml by the 9th week after infection. Six weeks later, the infected calves regained values in excess of 6.0 gm/100 ml. The author believed that the hypoproteinemia in the parasitized calves may have resulted from a greater intestinal loss of plasma relative to erythrocytes. Lowering of protein level in case of Ancylostomiasis has also been reported by Gilles et al. 1964, Nath et al. 1964 and the state of hypoproteinemia in such cases appeared to be due to the exudation of plasma through the diseased mucosa.

Observations of Singh et al. (1971) on the serum protein changes in Ancylostomiasis brought some logical explanation for this controversial issue. In the beginning, total protein was lower in all patients as compared to that of normal. Treatment of anaemia in patients with hookworm disease produced a significant rise of total proteins. It was found that deworming

caused a reversal of these changes to normal thereby suggesting direct influence of the infection.

In brief, it could be concluded that the increase in the protein level in one case and decrease in the other is related to 3 factors (i) maturity of worms, (ii) development of active immunity and (iii) exudation of plasma from the diseased tissue. Absence of any one factor may influence the protein contents accordingly. So differential diagnosis based on the protein changes must be considered in the light of the above factors.

(b) Albumin and globulin:

Experimental procedure:

Albumin and globulin were estimated quantitatively by Greenburg method.

Reagents: (1) 22.5% sodium sulphate solution - 22.5 gm of reagent grade sodium sulphate was transferred in an volumetric flask (100 ml). The substance was dissolved first with a little distilled water then it was filled till 100 ml mark with distilled water.

(2) 5 N sodium hydroxide solution - 2 gm of sodium hydroxide was dissolved in 100 ml distilled water in an volumetric flask.

(3) Standard tyrosine solution - 20 mg of tyrosine was dissolved in 100 ml of 0.1 N hydrochloric acid in an volumetric flask.

(4) Folin phenol reagent.

0.5 ml of serum was taken in a tube. 9.5 ml of 22.5% sodium sulphate was added. The solution was agitated thoroughly and kept in an incubator at 37°C for 2 hours to allow coagulation of globulins. After two hours the content was filtered using a retentive filter paper. The filtrate was used for albumin analysis.

For the determination of albumin a 5 cc aliquot of the filtrate was taken into a 50 ml volumetric flask, about 25 ml water was added. The other reagents were added as follows:

Reagent	Blank (ml)	Sample in ml			
		1	2	3	4
Aliquot	-	5	5	5	5
5 N NaoH	2	2	2	2	2
Folin Phenol Reagent	3	3	3	3	3

All the flasks were filled to 50 ml mark with distilled water. The colour was read in photometer after 5-10 minutes at 540 mμ.

Globulin.

The precipitate of the above experiment was analysed for globulin determination. The precipitate was washed twice

with 3 ml each of sodium sulphate. The funnel along with filter paper containing the precipitate was transferred to a 50 ml volumetric flask. A hole was punctured in filter paper and the precipitate was washed with a stream of approximately 0.01 N sodium hydroxide from a wash bottle. The washing was completed with distilled water until the flask is half filled. In the flask were added

Reagents	Blank (ml)	Samples in ml			
		1	2	3	4
Aliquot	-	5	5	5	5
5 N NaoH	2	2	2	2	2
Water	25	25	25	25	25
Folin Phenol Reagent	3	3	3	3	3

All the flasks were filled to 50 ml mark with distilled water. They were kept for 5-10 minutes for colour development. The colour was read in photometer setting the photometer to zero with blank at 540 mμ. Standard curve was plotted earlier with tyrosine solution using the same reagents.

Results and discussion:

Albumin and globulin are the main constituents of total protein. In normal serum the albumin level is higher than the globulin. The albumin and globulin values were estimated quantitatively both in normal and infected rats (Table 1).

The results (Fig. 1) shows a gradual increase of globulin and reciprocal decrease of albumin. The mean globulin level in the normal rats was 3.1 gm/100 ml after the 4th week of infection, the globulin level reached to mean value of 4.84 gm/100 ml. The mean albumin value in the normal rats was 2.22 gm/100 ml and after the infection it started decreasing and reached to a minimum mean value of 1.24 gm/100 ml. As a result albumin/globulin ratio was also got disturbed. The albumin/globulin ratio decreased to a mean value of 0.25 from the normal mean value of 0.71. Berghen (1966) studies on serum protein changes in Capillaria obsigmata infection also presented hypoalbuminemia accompanied with hyperglobulinemia. Data obtained from the third and fourth week after infection reflected a progressive and definite increase of globulin. A/G ratios also decreased sharply but albumins showed very minor fluctuations. The increase of globulin is a sign of active immunity. Disturbance of albumin/globulin ratio is a sign of pathological condition. .

Synder et al. (1967) studies on the blood chemical and cellular changes in canine Dirofilariasis also showed an increase of globulin and decrease of albumin in infected dogs.

Findings of Singh et al. (1971) on serum protein changes in Ancylostomiasis, Singh et al. (1972 and 1973) on the blood chemical changes in buffaloes infected with microfilaraemia, Sansom et al. (1974) on the Trichuris suis infection in weaner

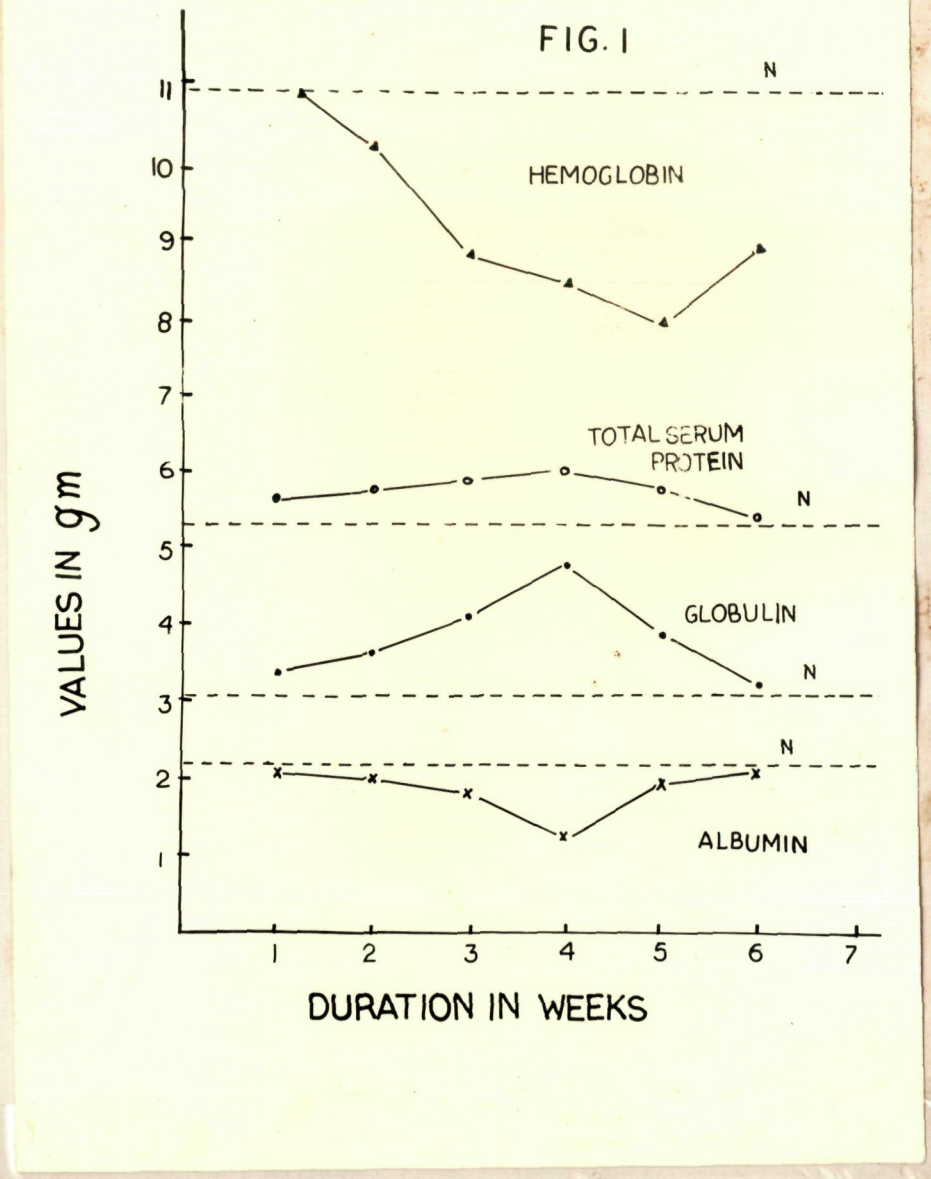

Fig. 1. Changes in proteins value in the blood of *S. cervi* infected rats.

pigs showed hyperglobuminemia and hypoalbuminemia which agreed with the present study. The fluctuation in protein fraction could be explained that after infection there may be some factor which inhibit the production of albumin. It may also be possible that due to hepatic damage liver was unable to convert the amino acids of food into serum albumin (as liver is the place of synthesis of albumin and other proteins except gamma globulin) resulting into hypoalbuminemia. Decline in serum albumin level may be due to impaired digestion of protein or inadequate absorption from the intestine. Since hypoalbuminemia is a characteristic diagnostic and prognostic sign in the liver diseases. Perhaps globulin increase may be a reason of local peritonitis by parasite resulting in an increase in globulin fraction in blood of rats. In pathology the change in the serum proteins for the most part affect the globulin. This increase is the major factor in causing the accelerated sedimentation rate of red cells. The change in albumin ratio is particularly pronounced in chronic infection accompanied by accumulation of antibodies which in their chemical structure belong to globulin. The increase of globulin is a sign of active immunity and disturbance of albumin/globulin ratio is a sign of pathological condition.

(c) Hemoglobin:

Experimental procedure:

Hemoglobin was determined by Wong principle.

Reagents: (1) Concentrated sulphuric acid (AR).

(2) Saturated potassium persulphate solution - shake 7-8 gm of reagent grade iron free potassium persulphate with 100 ml of water in a glass stopper bottle. The undissolved excess content settles to the bottom and compensate for loss by decomposition. The solution was kept in refrigerator.

(3) 10% sodium tungstate solution - 10 gm of reagent grade sodium tungstate was dissolved in 100 ml water in volumetric flask.

(4) Standard iron solution - 0.702 gm of reagent grade crystalline ferrous ammonium sulphate (Mohr's salt) was dissolved in 100 ml of water. 5 ml of concentrated sulphuric acid was added. The solution was warmed and in it was added potassium permagnate solution drop by drop until one drop oroduces a permanent colour. Transferred to a 1 liter flask with rinsing, diluted to the mark with distilled water and mixed. This solution contained 0.1 mg of ferric iron/ml and was stable indefinitely.

(5) 3 N potassium thiocyanate solution - dissolved 146 gm of reagent grade potassium thiocyanate in water and diluted to 500 ml. Filtered if turbid. 2 ml of acetone was added to improve the keeping quality.

With an Ostwald or micropippet accurately transferred 0.5 ml of mixed oxalated whole blood to a 50 ml volumetric flask. 2 ml of iron free concentrated sulphuric acid was added. The mixture was mixed with whirling. 2 ml of saturated potassium persulphate was added in it. Mixed and diluted to about 25 ml mark with water, followed by 2 ml of 10% sodium tungstate. Mixed, cooled to room temperature and diluted to the 50 ml mark with water. Filtered through a dry filter paper, collecting the filtrate in a dry flask.

A standard was prepared in a second 50 ml volumetric flask by adding about 25 ml water followed by 2 ml of concentrated sulphuric acid, 2 ml of saturated potassium persulphate solution and 2.5 ml standard iron solution containing 0.1 mg of ferric iron/ml. Cooled to room temperature, diluted with water to the mark and mixed.

Blank was prepared similar to the standard except that the standard iron solution was omitted.

10 ml of unknown filtrate, standard and blank were measured in separate tubes. To each was added 0.5 ml of saturated potassium persulphate solution followed by 2 ml of 3 N potassium thiocyanate solution.

The content were mixed and the colour was read after 30 minutes in a photometer, setting the photometer to zero density with blank at 480 mμ. The values were calculated by the following formula.

$$\frac{\text{Density of unknown}}{\text{Density of known}} \times 0.25 \times \frac{100}{0.5} \times \frac{1}{3.4}$$

Results and discussion:

The most important constituents of red blood cell is the compound hemoglobin (Hb) contained in its interior. One third of the red blood cell is comprised of hemoglobin. The hemoglobin values were determined both in normal and infected rats (Table 1). The data presented by Figure 1 shows a decrease in hemoglobin level during the infection period. The normal rats had mean hemoglobin value of 10.95 gm% while after the implant of Setaria cervi worms the hemoglobin level decreased to 8.46 gm%. More or less similar pattern was presented by Synder et al. (1967) in canine dirofilariasis, Bremmer (1969) in bovine oesophagostomiasis and Singh et al. (1972 and 1973) in buffaloes with microfilariasis. Decrease in hemoglobin level can result either from an excessive loss or destruction of red cells. This destruction may be produced by the toxic metabolites released by the parasite. This toxic factor cause a hemolysis and increase erythrocyte destruction. A defective formation of new cells, due to damage of red cell forming organ may also be one of the reasons of low hemoglobin in infected animals.

Table 1. Total protein, albumin, globulin, albumin/globulin ratio and hemoglobin in normal and infected rats.

OBSERVATIONS	TOTAL PROTEIN gm%	ALBUMIN gm%	GLOBULIN gm%	A/G RATIO	HEMOGLOBIN gm%
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Normal	5.32 ± 0.315	2.22 ± 0.011	3.10 ± 0.01	0.71 ± 0.021	10.95 ± 0.01
After 1 week of infection	5.65 ± 0.065	2.21 ± 0.01	3.44 ± 0.021	0.64 ± 0.005	10.93 ± 0.01
After 2 week of infection	5.77 ± 0.015	2.09 ± 0.005	3.68 ± 0.005	0.56 ± 0.004	10.22 ± 0.075
After 3 week of infection	5.93 ± 0.031	1.82 ± 0.012	4.11 ± 0.011	0.44 ± 0.064	8.89 ± 0.06
After 4 week of infection	6.08 ± 0.023	1.24 ± 0.0	4.84 ± 0.0	0.25 ± 0.026	8.46 ± 0.08
After 5 week of infection	5.88 ± 0.035	1.95 ± 0.013	3.93 ± 0.012	0.49 ± 0.065	7.95 ± 0.18
After 6 week of infection	5.44 ± 0.12	2.15 ± 0.005	3.29 ± 0.006	0.63 ± 0.005	9.41 ± 0.17

2. Glucose

Experimental procedure:

Glucose was estimated by Folin Wu Method. Protein from the blood was precipitated by Somogyi Method.

Protein precipitation:

Reagents:- (1) 10% solution of zinc sulphate - 10 gm reagent grade zinc sulphate was dissolved in distilled water in a 100 ml volumetric flask. The flask was filled to its mark with distilled water.

(2) 0.5 N sodium hydroxide - normal sodium hydroxide was diluted with equal volume of boiled distilled water.

To 1 volume of whole blood 7 volume of distilled water was added. Then 1 volume of zinc sulphate was added and mixed. 0.5 N sodium hydroxide was added. Tubes were shaked well and filtered.

Glucose determination:

Reagents:- (1) Alkaline copper solution - dissolved 40 gms of sodium carbonate in 400 ml of distilled water. Dissolved 11.5 gms of cupric sulphate and in 200 ml of distilled water, dissolved 7.5 gms of tartaric acid in 300 ml of distilled water. In each case the solutions were heated to hasten

the solution. Cooled to room temperature. Then the tartaric acid solution was pured into carbonate solution and cupric sulphate solution was added in it. Diluted to 1 liter.

(2) Phosphomolybdic acid solution - 40 gms of sodium hydroxide was dissolved in 400 ml of distilled water. 70 gms of molybdic acid, 10 gms of sodium tungstate and 400 ml of distilled water was added to it. Boiled for half an hour. Cooled, diluted to 700 ml. To it was added 250 ml of phosphoric acid and diluted to 1 liter.

(3) Benzoic acid - dissolved 2.5 gms of benzoic acid in 1 liter of hot distilled water and cooled.

(4) Standard sugar solution (stock) - weighed 1 gm of pure dry glucose. Transferred to a 100 ml volumetric flask and filled to the mark with benzoic acid solution.

Working standard - 5 ml of stock solution was transferred into a 500 ml volumetric flask and diluted to the mark with benzoic acid solution.

1. 2 ml of protein free filtrate was pipetted into a 25 ml volumetric flask.

2. To a similar flask was added 2 ml of dilute standard of sugar solution.

3. 2 ml of distilled water was taken in a third flask.

4. To each flask was added 2 ml of alkaline copper solution.

5. Flasks were transferred to a boiling water bath for 8 minutes.

6. Cooled for 2-3 minutes without shaking.

7. To each flask was added 2 ml of phosphomolybdic acid solution.

8. After 2-3 minutes the flasks were filled to the mark with distilled water.

9. After 10 minutes the colour was read in a photometer, setting the photometer to zero density with blank at 520 mu.

Results and discussion:

The carbohydrates of food are absorbed as hexose, glucose, fructose and galactose. After absorption these sugars are carried by the portal circulation to the liver where a large proportion is absorbed and converted into glycogen. Maintenance of normal concentration of glucose in the blood and tissue depends upon the breakdown of glycogen into glucose and its orderly release by the liver.

The glucose values were estimated quantitatively both in normal and infected rats (Table 2). Data (Fig. 2) shows that due to Setaria cervi infection a gradual increase was observed and at the peak of the infection the value was almost double (131.15 mg%) as compared to the normal value (99.4 mg%). Pierce et al. (1939) studies on human trichinosis also presented hyperglucaemia. Similar findings were obtained by Synder et al. (1967)

in canine dirofilariasis, Singh et al. (1972 and 1973) in buffaloes with microfilariasis. This marked increase could be interpreted due to the liver damage by the parasite. Since Setaria cervi worms after transplant have also been found to force their way into this organ and penetrate into the bile passages and interior tissues. Because of this hepatic disorders either the liver is unable to store much glucose as glycogen or oxidation gets disturbed somehow. It is removed from the blood very slowly and as a result the blood sugar rises rapidly to a much higher level. The hyperglucaemia may also be due to less utilization of glucose by the tissues.

Table 2. Glucose values in normal and infected rats.

OBSERVATIONS	GLUCOSE mg% Mean ± S.E.
Normal	99.4 ± 2.25
After 1 week of infection	101.8 ± 2.45
After 2 week of infection	118.7 ± 1.85
After 3 week of infection	128.3 ± 2.65
After 4 week of infection	131.15 ± 2.865
After 5 week of infection	119.7 ± 2.5
After 6 week of infection	106.55 ± 1.95

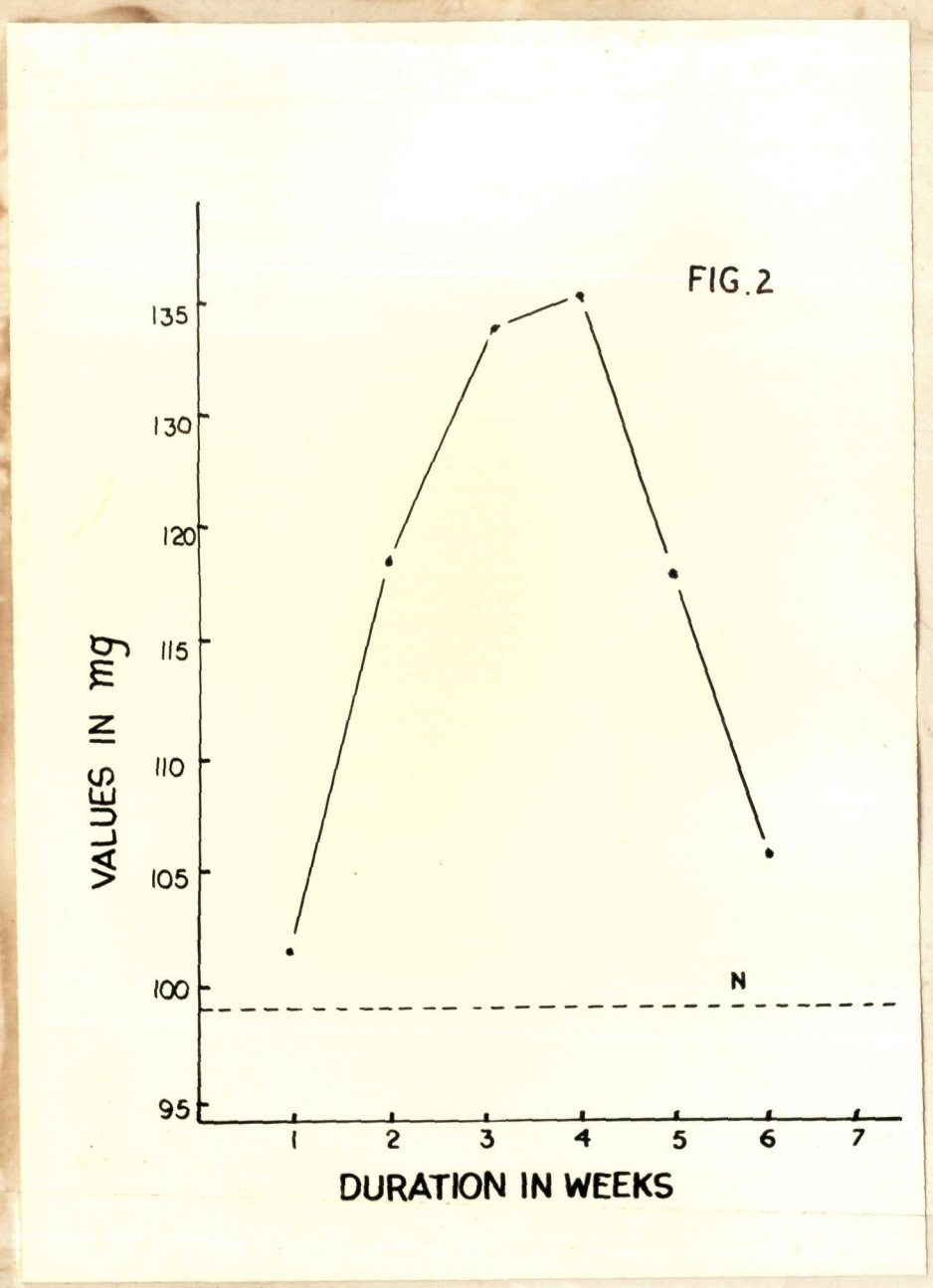

Fig. 2. Changes in glucose value in the blood of S. cervi infected rats.

3. Cholesterol

Experimental procedure:

The cholesterol was determined by Reinhold and Shiels method.

Reagents:- (1) Anhydrous sodium sulphate

(2) Chloroform (distilled)

(3) Acetic anhydride sulphuric acid reagent - just before use 1 volume of concentrated sulphuric acid was added to 10 volume of acetic anhydride. Discarded after the use.

(4) Cholesterol standard (stock) - 50 mg of pure dry cholesterol was dissolved in chloroform in 100 ml volumetric flask, diluted to the mark with chloroform.

Working standard - 5 ml of stock standard was transferred to 100 ml volumetric flask and diluted to the mark with chloroform. This solution contains 0.8 mg of cholesterol in 10 ml.

1 ml of serum was transferred to a mortor containing 8 gm anhydrous sodium sulphate. Dried in an oven at 100°C for overnight. Cooled in a desiccator, pulverized and transferred completely to a paper extraction cell which is then inserted into a soxhlet extraction tube. Placed 20-25 ml redistilled chloroform in the extraction flask, placed on an electric hot

plate and extracted for 3 hours during which time the cold water flowed through the condensor of the coil. Allowed the extract to cool and transferred with rinsing to a 25 ml volumetric flask. Diluted to the mark with chloroform mixed well and further proceeded as follows:

Reagents	Blank (ml)	Standard (ml)	Samples in ml			
			1	2	3	4
Aliquot	-	-	10	10	10	10
Standard cholesterol	-	10	-	-	-	-
Chloroform	10	-	-	-	-	-
Acetic anhydride sulphuric acid reagent	2	2	2	2	2	2

All the tubes were kept in dark for 30 minutes. The colour was read in photometer, setting the photometer to zero with blank at 660 mμ.

Results and discussion:

Cholesterol levels were determined both in normal and infected rats (Table 3). Data (Fig. 3) shows a decrease of cholesterol in infected rats. The rats after infection had a mean value of cholesterol 139.23 mg% as compared to the normal rats which had mean value of 153.9 mg%.

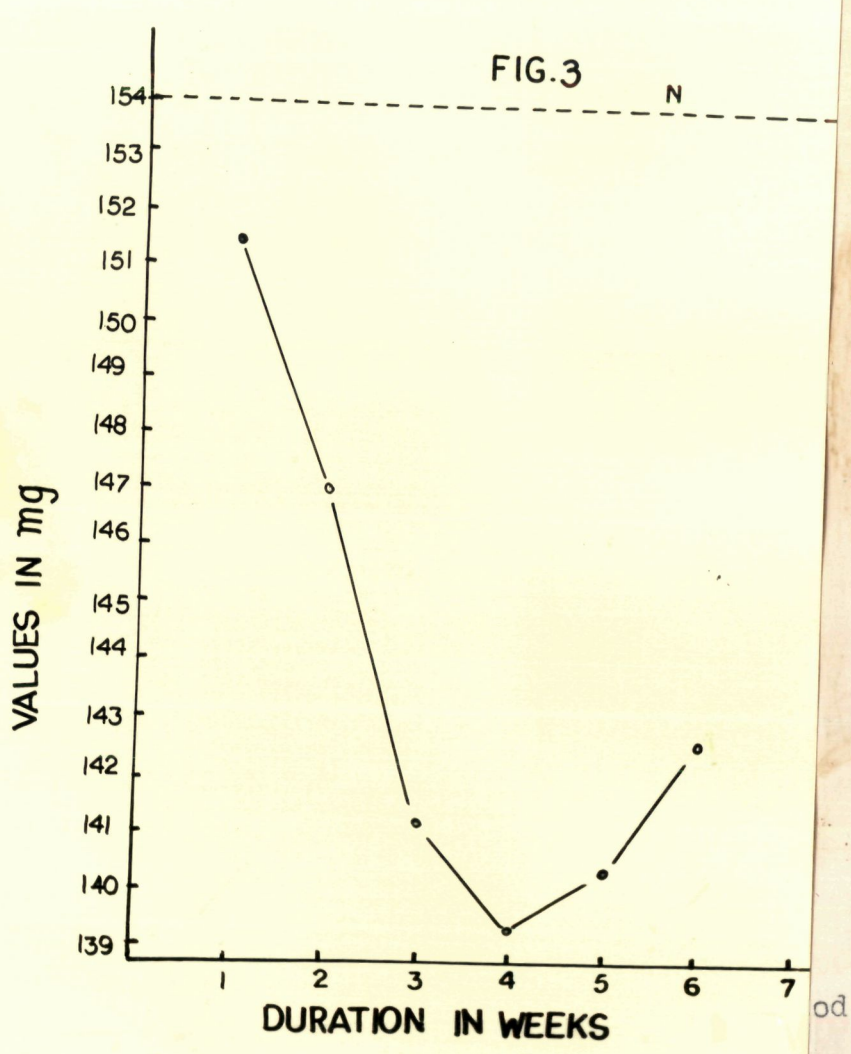
FIG.3
N
154
153
152
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150
149
148
147
146
145
144
143
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141
140
139
VALUES IN mg
1
2
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4
5
6
7
DURATION IN WEEKS

Fig od of

A fall in serum cholesterol in patients with Ancylostomiasis was reported by Nayar (1942) and Hand (1963). Studies of Singh et al. (1971) also indicated a decrease of cholesterol level. Treatment of patients with drugs or deworming showed an increase approaching to the normal level. It is presumed that the worms were in some way responsible for this change. It might be that the presence of parasite caused an inhibition of normal synthesis of certain lipid factors particularly cholesterol or increased the consumption of lipids by the parasites themselves.

Table 3. Cholesterol values in normal and infected rats.

OBSERVATIONS	CHOLESTEROL mg% Mean ± S.E.
Normal	153.9 ± 2.82
After 1 week of infection	151.45 ± 2.445
After 2 week of infection	147.09 ± 2.19
After 3 week of infection	141.17 ± 3.06
After 4 week of infection	139.23 ± 2.81
After 5 week of infection	140.20 ± 4.53
After 6 week of infection	142.53 ± 3.92

4. Minerals

(a) Calcium:

Experimental procedure:

Calcium was determined by Roe and Kahn method.

Reagents:- (1) 10% Trichloroacetic Acid (TCA)

(2) 25% Sodium hydroxide

(3) 5% Trisodium phosphate

(4) Alkaline alcohol wash reagent - 85 ml of ethyl alcohol was mixed with 10 ml of amyl alcohol and diluted to 100 ml with water. 2 drops of 1% phenopthaline was added in it and then 5% NaoH was added one drop at a time to a distinct pink.

(5) Acid molybdate reagent - 12.5 gm of reagent grade ammonium molybdate was dissolved in 400 ml of water in a 500 ml volumetric flask. 100 ml of concentrated sulphuric acid was added slowly drop by drop in it.

(6) Aminonapthol sulfonic acid reagent (ANSA) - 195 ml of 15% sodium bisulphite was placed in a glass stopper cylinder. Added 0.5 gm of 1, 2, 4, Aminonapthol sulfonic acid in it. 5 ml of 20% sodium sulphide was added. Stoppered and shaked until the powder was dissolved. If solution was incomplete, more sodium sulphite was added (1 ml at a time) but excess was avoided. The solution was transferred to a brown bottle and stored in

the cold. This solution was usable for 4 weeks if kept under these conditions.

(7) Standard phosphate solution (stock) - 2.265 gm of pure dry monopotassium phosphate was dissolved in water diluted to 1 liter. A little chloroform was added as a preservative. This stock solution contains 0.517 mg of phosphorus/ml equivalent to 1 mg of calcium as calcium phosphate.
Working standard - 1 ml of stock solution was diluted to 100 ml with water. The standard should be prepared fresh daily.

In 8 ml of 10% TCA in a small flask added 2 ml of serum. Mixed well by shaking. Filtered through a calcium free filter paper. Transferred 5 ml of filtrate to a 15 ml conical graduated centrifuge tube, 1 ml of 25% calcium free sodium hydroxide solution was added and allowed to stand 5 minutes. 1 ml of 5% trisodium phosphate was then added, mixed thoroughly and set aside for 1 hour. Centrifuged for 2 minutes supernatant liquid was decanted, place the tube in an inverted position in a small beaker containing a filter paper and allow to drain 2 minutes. 5 ml of alkaline alcoholic wash reagent was added, a stirring rod was used to break up the precipitate. Washing and centrifugation was repeated for a second time with 5 ml of alcoholic wash reagent. After wiping off excess fluid the precipitate was dissolved in 10 ml of water, and set aside for few minutes. A standard was prepared by transferring 10 ml of standard phosphate solution in a second

tube. For photometric measurement a third tube or blank was prepared by taking 10 ml of distilled water.

When all the tubes were ready 1 ml of acid molybdate was added. After 2-3 minutes 0.5 ml of ANSA was added, followed by water to 15 ml mark and mixed immediately by inversion. The colour was read after 10 minutes in a photometer, setting the photometer to zero density with blank at 660 mμ.

(b) Inorganic phosphate:

Experimental procedure:

Inorganic phosphate was determined by Fisk and Subbarow method.

Reagents:- (1) 10% TCA

(2) 10 N sulphuric acid - 450 ml of concentrated sulphuric acid was mixed with 1300 ml of water.

(3) Molybdate I - 25 gm of reagent grade of ammonium molybdate was dissolved in about 200 ml of water. In 1000 ml volumetric flask, was placed 500 ml of 10 N sulfuric acid. Added the molybdate solution and diluted with washings to 1 liter with water.

(4) Molybdate II - 25 gm of reagent grade ammonium molybdate was dissolved in about 200 ml of water. In 1 liter volumetric flask placed 300 ml of 10 N sulphuric acid and added molybdate solution in it. Diluted with washing to 1 liter with water.

(5) ANSA reagent - (As in the previous experiment page no.32).

(6) 15% sodium bisulfite solution - 15 gm of sodium bisulfite was dissolved in 100 ml of water. If turbid allowed to stand for several days and filtered. Kept well stoppered.

(7) 20% sodium sulfite solution - dissolved 20 gm of sodium sulfite in 100 ml of water. Filtered if necessary. Kept well stoppered.

(8) Standard solution - exactly 0.351 gm of pure dry monopotassium phosphate was dissolved in water and transferred quantitatively to a 1 liter volumetric flask. 10 ml of sulfuric acid was added, diluted to the mark with water and mixed. This solution contained 0.4 mg of phosphorus in 5 ml.

To 8 ml of 10% TCA solution was added slowly with mixing 2 ml of whole blood, stoppered, shaked and filtered. Filtrate was used for the analysis of inorganic phosphorus.

Reagents	Blank (ml)	Standard (ml)	Samples in ml			
			1	2	3	4
Aliquot	-	-	5	5	5	5
Standard phosphate	-	5	-	-	-	-
TCA	5	-	-	-	-	-
Molybdate II	1	1	1	1	1	1
ANSA	0.5	0.5	0.5	0.5	0.5	0.5

All tubes were diluted to the 10 ml mark with distilled water. Allowed to stand for 5 minutes. Read in a photometer at 660 or 720 mμ.

Results and discussion:

Calcium occurs in serum in two forms, one part which appears to be physiologically inactive and is kept in solution by a combination with plasma protein and is diminished in conditions in which plasma protein is diminished. The balance which is physiologically active calcium behaves as if it existed in plasma as $Ca_3(Po_4)_2$ in saturated solution in contact with undissolved $Ca_3(Po_4)_2$ (in bone). This calcium is only clinically significant. Determination of the calcium in the serum is of considerable diagnostic significance. In the present experiment calcium and phosphorus levels were estimated quantitatively both in normal and infected rats (Table 4). The data (Fig. 4) presents a marked hypocalcaemia and hypophosphaemia in the infected rats. Normal rats had a mean value of calcium 9.22 mg% and a mean value of phosphorus 5.12 mg%. After the infection the calcium and phosphorus levels decreased and it reached to a mean value of 5.98 mg% and 3.04 mg%.

Studies of Pierce (1939) on human trichinosis, Synder et al. (1967) in canine dirofilariasis and Singh et al. (1972 and 1973) on buffaloes with microfilariasis showed the same findings. This imbalance of the minerals could be explained in the light

of metabolism. In the body, either of the element is deficient (as in the present case) and normal deposition of calcium and phosphate in ostoid tissue does not occur. Due to this imbalance in the metabolism the amount of calcium and phosphorus in the faeces is high resulting to hypocalcaemia and hypophosphaemia.

(c) Potassium:

Experimental procedure:

Potassium was determined by the Looney and Dyer method.

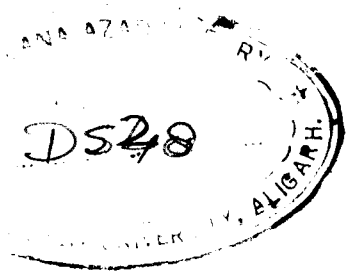
Reagents:- (1) Sodium tungstate 1.5%.

(2) Copper sulphate 2.5%.

(3) Silver nitrate 2.5%.

(4) Standard potassium solution (stock) - a stock standard is prepared by dissolving 2.229 gms of pure dry potassium sulphate in water in 1 liter volumetric flask, diluted with water to the mark and mixed. Preserved with a little tolvene. This solution contained 1 mg of potassium/ml and was stable indefinitely.

Working standard - 1 ml of stock solution was diluted to 100 ml with distilled water in an volumetric flask. This solution contained 0.03 mg of potassium in 3 ml.



(5) Silver cobaltnitrite reagent - Silver cobaltnitrite reagent was prepared as follows:

A. Dissolved 25 gms of cobaltnitrite in 50 ml of water and added 12.5 ml of acetic acid.

B. Dissolved 120 gms of reagent grade sodium nitrite in 180 ml of water.

210 ml of B reagent was added to all of A reagent. Air was thrown through the solution until all of the nitrous oxide fumes had been driven off. This solution was stable for one month if stored in refrigerator. It must be filtered each time before using. To prepare the silver cobaltnitrite reagent 1 ml of 40% silver nitrate was added to 20 ml of filtered sodium cobaltnitrite solution. shaked well and filtered. Prepared fresh for each series of analysis.

(6) Wash reagent - Mixed 2 volume of 95% ethyl alcohol with 1 volume of ether and 2 volume of water.

(7) 50% Hcl - Mixed 1 volume of water and 1 volume of concentrated hydrochloric acid.

(8) Sulfanilamide solution - 0.5 gm of pure sulfanilamide powder was dissolved in a mixture of 30% glacial acetic acid and 70 ml of water. Prepared fresh weekly.

(9) Napthylethylendiamine reagent - 0.1 gm of N-(1-napthyl)ethylediamine hydrochloride was dissolved in a mixture of 30 ml of acetic acid and 70 ml of water. Prepared fresh weekly.

Transferred 0.5 ml of serum to a small clean tube containing 7 ml of distilled water, added 1 ml of 15% sodium tungstate. Mixed by tapping and followed with 1 ml of 2.5% copper sulphate solution. Stoppered and shaked well then added in it 0.5 ml of 2.5% silver nitrate solution. Stoppered and shaked again, then allowed to stand 15-20 minutes. Poured on a dry filter paper, returning the first portion of the filtrate to the funnel to ensure obtaining a clear filtrate.

1. Transferred 3 ml of filtrate to a clean centrifuge tube.
2. In a second tube 3 ml of standard potassium solution was taken containing 0.03 mg of potassium.
3. 1 ml of 95% alcohol and 1 ml of water was added in it.
4. Mixed by tapping and replaced in water bath at 18 or 22°C for 5 minutes.
5. 2 ml of freshly prepared and filtered silver cobaltnitrite reagent, mixed by tapping and replaced in the water bath for 2 hours.
6. Centrifuged carefully, removed the supernatant fluid down to 0.2 ml mark with a capillary pippet.
7. Added 7 ml of wash reagent down to the side of the tube, disturbing the precipitate as little as possible. Centrifuged for 15 minutes and drained.
8. Washing and draining was repeated second time.
9. The precipitate was dissolved in 10 ml of 0.2 normal sodium hydroxide.

10. The tubes were placed in a boiling water bath for 10 minutes.
11. Mixed well and centrifuged.
12. Added 2 ml of supernatants to 100 ml volumetric flask. In a third volumetric flask 2 ml of water was taken for blank.
13. Added 5 ml of water followed by 1 ml of 50% Hcl and 2 ml of 0.5% sulfanilamide solution.
14. Mixed by lateral shaking allowed to stand for 3 minutes.
15. Added 1 ml of napthylethylenediamine reagent, diluted with water to 100 ml mark.
16. After 5 minutes the colour was red in photometer setting the photometer to zero density with blank at 520 mμ.

The values were calculated by the following formula.

$$\frac{\text{Density of unknown}}{\text{Density of known}} \times 0.03 \times \frac{10}{3} \times \frac{100}{0.5} = \text{mg of potassium/ 100 ml of serum}$$

Results and discussion:

Blood serum contains much less potassium than do erythrocytes. Potassium values were obtained quantitatively both in normal and infected rats (Table 4). Data (Fig. 4) shows a decrease of potassium level in infected rats. This agrees with Singh et al. (1972 and 1973) findings in buffaloes with microfilariasis. As potassium metabolism is closely connected to carbohydrate metabolism, in case of increase glycogenesis in the liver and muscles the potassium content in the serum is decreased. Potassium is eliminated through the kidneys, its elimination

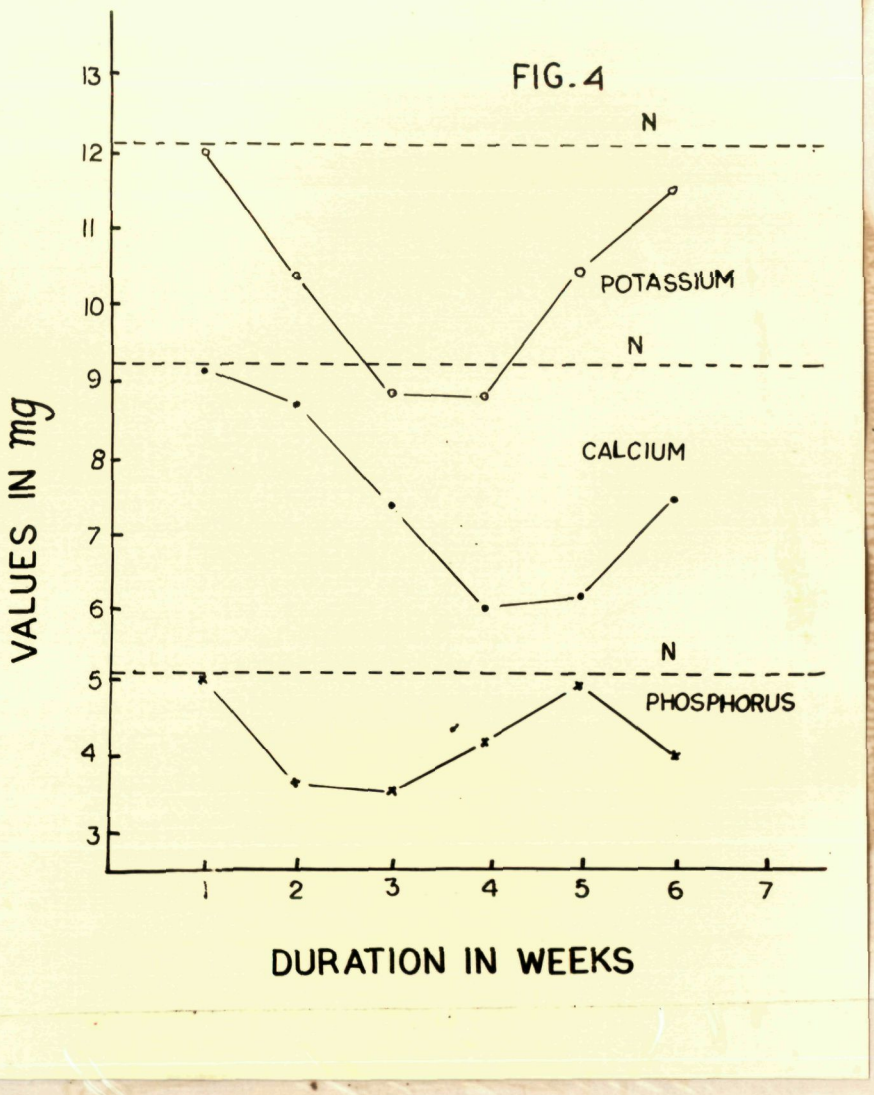


Fig. 4. Changes in calcium, phosphorus and potassium values in the blood of S. cervi infected rats.

being connected with that of water. Potassium deficiency is characterized by muscular weekness. Potassium metabolism is regulated by adrenals. Decrease in potassium level may also be due to Aldosterone which mobilizes potassium and caused its increased excretion in the urine by inhibiting its reabsorption in the kidneys.

Table 4. Calcium, phosphorus and potassium values in normal and infected rats.

OBSERVATIONS	CALCIUM mg% Mean ± S.E.	PHOSPHORUS mg% Mean ± S.E.	POTASSIUM mg% Mean ± S.E.
Normal	9.22 ± 0.047	5.12 ± 0.115	12.14 ± 0.094
After 1 week of infec.	9.12 ± 0.177	4.97 ± 0.04	12.04 ± 0.18
After 2 week of infec.	8.65 ± 0.055	3.65 ± 0.024	10.35 ± 0.13
After 3 week of infec.	7.32 ± 0.089	3.04 ± 0.013	8.8 ± 0.095
After 4 week of infec.	5.98 ± 0.025	4.16 ± 0.017	8.74 ± 0.11
After 5 week of infec.	6.14 ± 0.039	4.93 ± 0.034	10.21 ± 0.031
After 6 week of infec.	7.42 ± 0.34	4.04 ± 0.0	11.50 ± 0.14

5. Phosphatases

(a) Alkaline phosphatase:

Experimental procedure:

Alkaline as well as acid phosphatase was determined by Bodansky method with a little modification of Fisk and Subbarow method.

Reagents:- (1) Alkaline phosphate substrate - Into a 100 ml volumetric flask introduced successively 3 ml of petrolium ether (B.P. 20-30°C), about 80 ml of distilled water, 0.5 gm sodium - glycerophosphate, 0.424 gm of sodium diethylbarbiturate and water to 100 ml mark. Transferred into a glass stoppered pyrex bottle containing an inch layer of Petrolium ether. Kept in the refrigerator.

(2) 30% TCA

(3) Standard phosphate solution - Placed 6.25 ml of standard phosphate solution (containing 0.5 mg of phosphorus) in a 100 ml volumetric flask. Added 16.7 ml of 30% TCA. Diluted to the mark with water. This solution contained 0.04 mg of phosphorus in 8 ml of TCA.

(4) Aminonapthol sulphonic acid reagent (ANSA) - prepared in the same way as prepared for the calcium (page no.32).

Measured 8 ml of alkaline phosphate substrate into a glass stoppered cylinder and placed in an incubator or water bath at 37°C until the tube reached the incubator temperature. 1 ml of serum was added after noting the time it was incubated for exactly one hour. Removed and cooled in ice water for several minutes and added 2 ml of 30% TCA. Mixed and let stand for few minutes and filtered through a low ash filter paper.

At or near the incubation period, prepared a control sample. Measured 9 ml of substrate into a glass stoppered cylinder and added 2 ml 30% TCA with mixing 1 ml of serum was added. Stoppered, shaked and filtered.

When both filtrates were ready 10 ml of these filtrates were transferred to each cylinder or graduated tubes. In a similar tube 8 ml of standard phosphate solution was placed, containing 0.04 mg of phosphorus. For photometric measurements blank was prepared by taking 8 ml of 30% TCA alone.

When all the tubes were ready added to each 1 ml of Molybdate II reagent and mixed. 0.4 ml of ANSA was added to each tube. Diluted immediately to 10 ml mark with water and mixed. Allowed to stand for 5 minutes for colour development.

The colour was read in a photometer, setting the photometer to zero density with blank at 660 mμ.

(b) Acid phosphatase:

Experimental procedure:

The procedure was exactly the same as for alkaline phosphatase. The unit of acid phosphatase actively being defined as equivalent to the liberation of 1 mg% of inorganic phosphate during 1 hour incubation at pH 5.0.

Reagents:- All reagents were the same as for alkaline phosphatase except the substrate.

Acid phosphate substrate:- This was also identical with the alkaline phosphate substrate already described except that sufficient acetic acid was incorporated to bring the pH to 5.0. Into a 100 ml volumetric flask, introduced successively 3 ml of petrolium ether, about 80ml of water, 0.5 gm of sodium - glycerophosphate, 0.424 gm of sodium diethylbarbiturate and 5 ml of 1 N acetic acid. Dissolved by mixing and added water to bring till 100 ml mark. The pH of the final solution should be checked and it should be 5.0.

Results and discussion:

Acid and alkaline phosphatases are the two main phosphatases of the blood. Historically this enzyme has been the subject of much clinical investigations in relation to diseases of liver. Acid and alkaline phosphatases were estimated quantitatively both in normal and infected rats (Table 5). Data (Fig. 6) shows that there is almost no change in acid phosphatase values, but an increase in alkaline phosphatase is

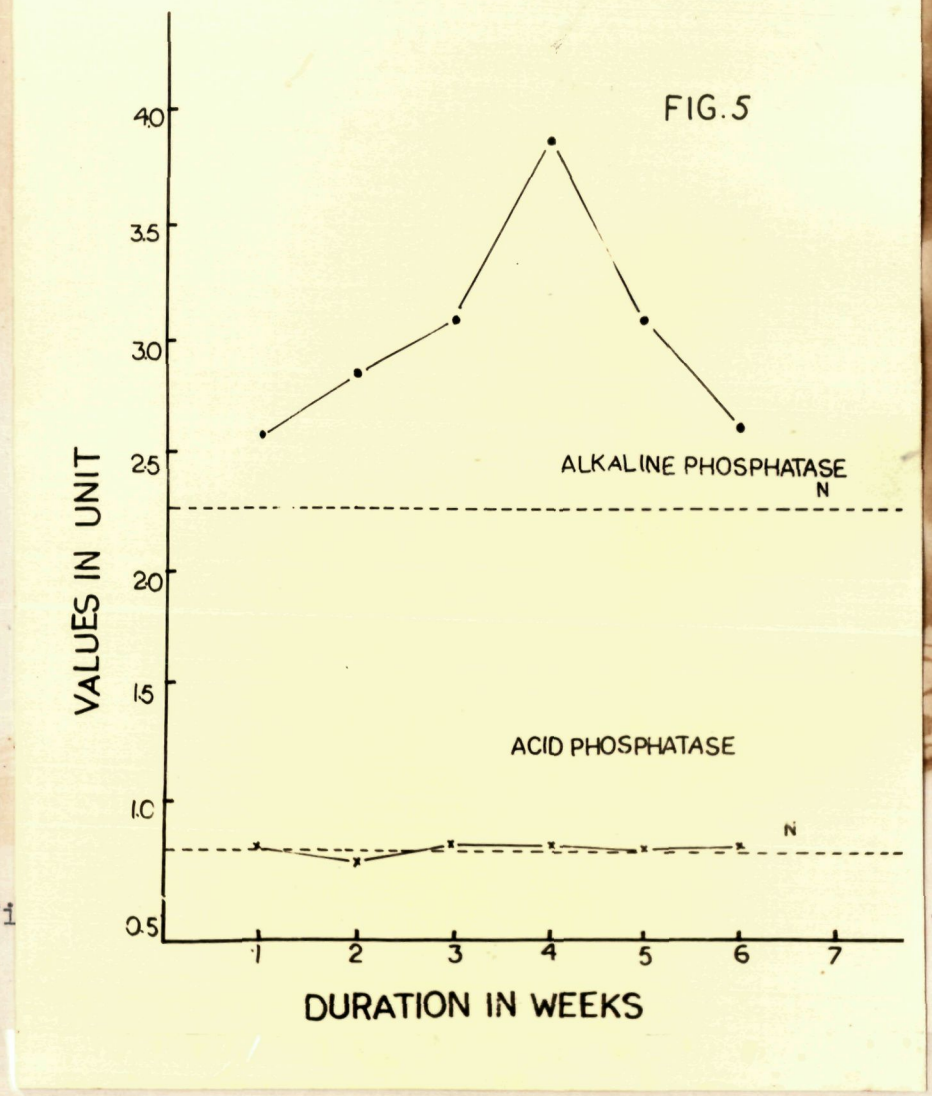


FIG. 5
VALUES IN UNIT
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ALKALINE PHOSPHATASE
N
ACID PHOSPHATASE
N
1
2
3
4
5
6
7
DURATION IN WEEKS

Fi values

indicated. The increase of alkaline phosphatase may be due to liver damage. It may also be due to hyperparathyroidism.

Table 5. Phosphatases values in normal and infected rats.

OBSERVATIONS	ACID PHOSPHATASE Unit %	ALKALINE PHOSPHATASE Unit %
	Mean ± S.E.	Mean ± S.E.
Normal	0.809 ± 0.045	2.308 ± 0.023
After 1 week of infection	0.802 ± 0.012	2.612 ± 0.0219
After 2 week of infection	0.749 ± 0.020	2.89 ± 0.045
After 3 week of infection	0.819 ± 0.012	3.107 ± 0.023
After 4 week of infection	0.812 ± 0.008	3.90 ± 0.04
After 5 week of infection	0.804 ± 0.011	3.13 ± 0.009
After 6 week of infection	0.811 ± 0.01	2.66 ± 0.035

6. Transaminases

(a) Serum glutamic oxalacetic transaminase (SGO-T)

(b) Serum glutamic pyruvic transaminase (SGP-T)

Experimental procedure:

Both these transaminases were determined by the method of Steinley Reitman and S. Frankel.

Reagents:- (1) Phosphate buffer - 0.1 MpH 7.4. Mixed 420 ml of 0.1 M Disodium phosphate and 50 ml of 0.1 M potassium dihydrogen phosphate.

(2) Pyruvate - 2 mM/liter (For standard curve). Dissolved 22.0 mgs of sodium pyruvate in 100 ml of phosphate buffer.

(3) - Ketoglutarate - 2mM/liter - dl aspartate 200 mM/liter (For SGO-T substrate and standard curve). Placed 29.2 mgs of -Ketoglutaric acid and 2.66 gms of dl aspartic acid in a small beaker. Added 1 N sodium hydroxide until the solution was complete. Adjusted to a pH of 7.4 with sodium hydroxide. Transferred quantitatively with buffer to a 100 ml volumetric flask and then diluted to the mark with buffer solution.

(4) 2,4,dinitrophenylhydrazine - 1 mM/liter. Dissolved 19.8 mgs of 2,4,dinitrophenylehydrozine in 100 ml of 1 N Hcl.

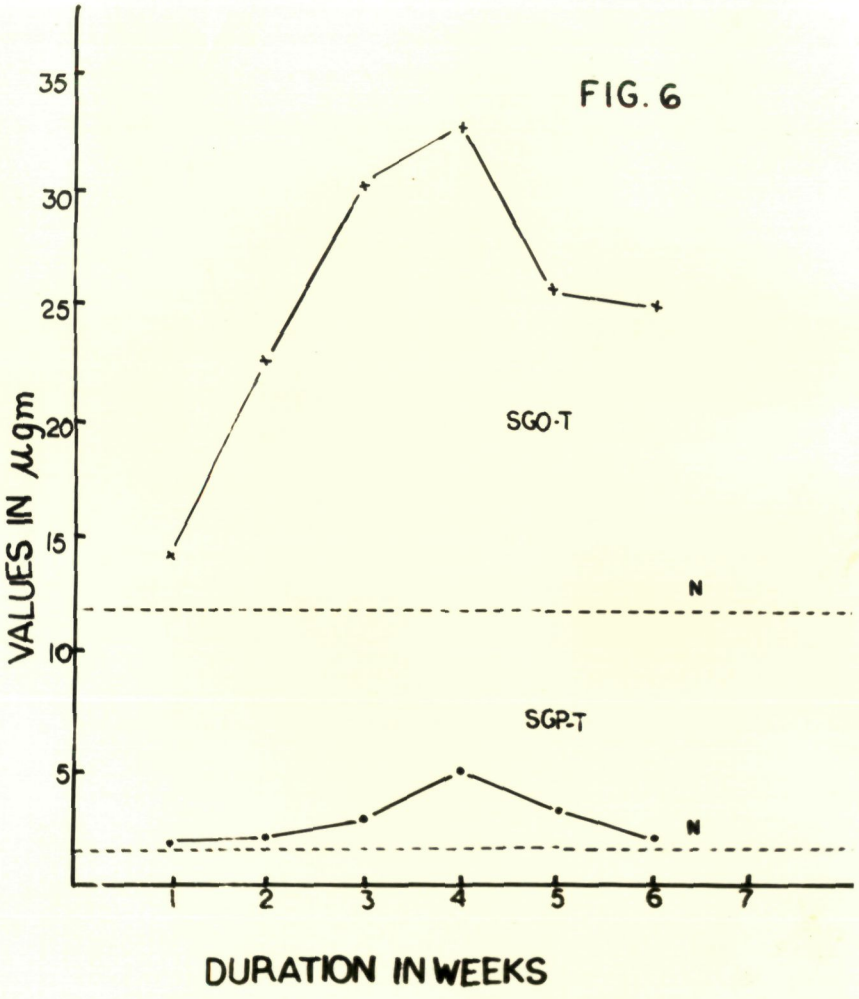


FIG. 6
35
30
25
20
15
10
5
VALUES IN µgm
SGO-T
N
SGP-T
N
1
2
3
4
5
6
7
DURATION IN WEEKS

Fig.

(5) Sodium hydroxide solution - 0.4 N sodium hydroxide solution was prepared.

1 ml of the substrate (-ketoglutarate for SGO-T and SGP-T) was pipetted into a test tube and placed in a water bath at constant temperature (40°C) for 10 minutes. 0.2 ml of serum was mixed to each and the contents were mixed. After an incubation period of exactly 30 minutes for SGP-T and 60 minutes for SGO-T, the tubes were removed from the water bath. 1 ml of 2,4, dinitrophenylehydrozine reagent was added immediately thereby stopping the reaction. The tubes were allowed to stand at room temperature for a minimum of 20 minutes. 10 ml of 0.4 N sodium hydroxide was added. Contents were mixed by inversion. At the end of exactly 30 minutes the optical density of the solutions were measured at 505 mu using water as blank.

While the specimens were incubating a control for each serum may be prepared. One ml of the substrate, 0.2 ml of serum and 1 ml of 2,4, dinitrophenylhydrozine reagents were mixed in a test tube. After a minimum of 20 minutes 10 ml of 0.4 N sodium hydroxide was added and the optical density of the coloured solutions were read in a photometer at 505 mμ.

Results and discussion:

Serum contains many different transminases. One of the most commonly determined is serum glutamic oxalacetic transminase 'SGO-T'. Another is serum glutamic pyruvic transminase 'SGP-T'.

The diagnostic value of the serum transminases in myocardial infarction and liver necrosis are now so well established that it has become one of the most reliable tests in clinical laboratory diagnosis. In the present experiment the level of SGO-T and SGP-T were estimated both in normal and infected rats (Table 6). The data (Fig. 6) shows that there is a gradual increase of these two enzymes. The value of SGO-T goes from 11.97 µg/ml to 32.88 µg/ml. While SGP-T goes from 1.432 µg/ml to 4.908 µg/ml. This agrees with Singh et al. (1972) findings in buffaloes with microfilariasis and Klunklin (1972) observations in Trichinae infection. This increase might be due to hepatocellular damage which increase the rate of release of enzymes into circulation. Increased permiability of cell membrane appears to account for the elevated serum levels of SGO-T. An increase in the tissue sources of enzymes, because of increased rate of production per cell or increased in the number of cells may be responsible for increased serum levels. In the tissue SGO-T is found in particularly high concentration in skeletal muscle, kidney and pancreas. Any factor affecting these tissues lead to increased serum levels of this enzymes. Elevated levels occur as early or earlier than other biochemical abnormalities.

Table 6. Transminases values in normal and infected rats.

OBSERVATIONS	SGO-T µg/ml	SGP-T µg/ml
	Mean ± S.E.	Mean ± S.E.
Normal	11.977 ± 0.74	1.432 ± 0.133
After 1 week of infection	14.21 ± 0.361	1.844 ± 0.26
After 2 week of infection	22.47 ± 0.57	2.065 ± 0.174
After 3 week of infection	30.272 ± 0.515	2.869 ± 0.021
After 4 week of infection	32.882 ± 0.275	4.908 ± 0.06
After 5 week of infection	25.87 ± 0.30	3.121 ± 0.37
After 6 week of infection	25.01 ± 0.25	2.01 ± 0.35

V. SUMMARY

Adult Setaria cervi worms collected from the freshly slaughtered cattles were transplanted via laprotomy, into the peritonial cavity of white rats to carry out investigations regarding blood chemical changes. Infection free rats were also maintained as control. Blood of infected rats was checked daily till the microfilariae were detected. Sample of blood from infected rats was drawn out at weeks interval and biochemical analysis was carried out.

Quantitative analysis of total serum protein, albumin, globulin, hemoglobin, glucose, cholesterol, calcium phosphorus, potassium, alkaline phosphatase, acid phosphatase, serum glutamic oxalacetic transminase and serum glutamic pyruvic transminase was carried out for a duration of six weeks.

An increase in total serum protein and globulin with low albumin level was observed in the infected rats. Increase of total protein was due to marked rise of globulin. Globulin increase may be a reason of local peritonitis by parasite resulting in an increase in globulin fraction in blood of rats. Hypoalbuminemia condition was related to hepatic damage and low hemoglobin due to loss of red cells.

Glucose level in the infected rats increased to almost double the quantity of the normal. This marked increase could be interpreted due to the liver damage by the parasite. Because of this hepatic disorders either the liver was unable to store much glucose as glycogen or oxidation got disturbed somehow. The hyperglucaemia may also be due to less utilization of glucose by the tissues.

A fall in serum cholesterol in infected rats could be correlated with the presence of parasite which caused an inhibition of normal synthesis of certain lipids particularly cholesterol. It may also be due to the increased consumption of lipids by the parasites themselves.

There was a decrease in calcium, phosphorus and potassium levels in infected rats. This imbalance was because of the increased amount of these salts in faeces.

There was almost no change in acid phosphatase value, but an increase in alkaline phosphatase was evident. The increase of alkaline phosphatase may be due to liver damage.

Both of the transminases, SGO-T and SGP-T increased in infected rats. The diagnostic value of the serum transminase in myocardial infarction and liver necrosis already so well established, it could be considered one of the reliable tests in parasitic infections also.

VI. REFERENCES

Bremner, K.C. 1969. Pathological factors in experimental bovine oesophogostomiasis II. Plasma iron, iron binding capacity and reticulocyte responses in blood of infected calves. Exp. Parasit. 24: 184-193.

Cyrus, H Fiske and Yellapragoda Subbarow, 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66: 375-400.

David M. Greenburg, 1929. The colorimetric determination of the serum proteins. Ibid. 82: 545-550.

Gilles, H.M., Watson, W.E.J. and Ball, P.A.J., 1964. Hookworm infection and anaemia. Quart. J. Med. 33: 1.

Hamann, C.B., 1943. Estimation of histamine in blood and other tissues of rats and guinea pigs infected with Trichinella spiralis. J. Parasit. 29: 367-372.

Hand, A.K., 1963. A study of plasma cholesterol free and esterified in anaemias. Thesis for M.D. Medicine submitted to Vikram University, Ujjain.

Hartman, E. and Pierce, et. al., 1939. Blood chemistry in human Trichinosis. Amer. J. Hyg. 29(2): 75-81.

Joseph, H., Roe and Bernard, S. Kahn, 1929. The colorimetric determination of blood calcium. J. Biol. Chem. 81: 1-8.

Klunklin, K., 1972. Serum transaminase and hepatic pathology in trichinosis. J. Med. Assoc. Thialand, 55(12): 695-700 (En.).

Lowry, O.H., RoserRough, M.J., 1951. Protein measurement with the Folin Phenol Reagent. J. Biol. Chem. 193: 265.

Nath, K., Kunwar, K.B. and Kulsreshtha, J.K., 1964. Electrophoretic pattern of serum protein in intestinal nematode infection. J. Ass. Phys. India, 12: 201.

Nayar, S., 1942. Cholesterol and anaemia. Indian Med. Gaz. 77: 459.

Otto Follin and Hsien Wu, 1920. A simplified and improved method for determination of sugar. J. Biol. Chem. 41: 367-375.

Otto Follin, 1926. The determination of sugar and in normal urine. Ibid., 76: 357-370.

Paul, Berghen, 1966. Serum protein changes in Capillaria obsignata infection. Exp. Parasit. 19(1): 34-42.

San Yin Wong, 1928. Colometric determination of iron and hemoglobin in blood II. J. Biol. Chem. 77: 409-412.

Sansom, B.F., Beer, R.J.S. and Kitchenham, B.A., 1974. Changes in the concentration of serum urea nitrogen, albumin, globulin, sodium and inorganic phosphorus in weener pigs infected with Trichuris suis. J. comp. Path. 84(3): 409-415 (En.).

Saraya, A.K., Tondon, B.N. and Ramchandran, 1970. A study of iron and protein deficiency in hookworm infection. Ind. Jour. Med. Res. (7-12): 1241.

Shastry, K.M.V. and Ahluwalia, S.S., 1972. Changes in serum proteins of goats experimentally infected with Haemonchus contortus. Ind. Vet. Jour. 49(5): 470-472(En.)

Singh, B., Mehta, S.K., Sarkar, A.K. and Chuttani, P.M., 1971. Serum proteins and lipid changes in the ancylostomiasis before and after treatment. Indian Jour. Med. Res. 59(4): 598-603.

Singh, D.V., Joshi, H.C. and Shivani, G.A., 1972. Blood chemical changes and chemotherapy of Buffaloes infected with Setaria cervi and Onchocerca armilata. Philipine Jour. Vet. Med., 9(2): 101-109.

Singh, D.V., Joshi, H.C. and Shivani, G.A., 1973. Biochemical and Haemotological studies in microfilariasis of buffaloes. Indian Jour. Exp. Biol. 11(4): 336-337.

Synder, J.S., Kwang, L. and Robert, J.T., 1967. Blood chemical and cellular changes in canine dirofilariasis. Amer. Jour. Vet. Res. 28: 1705.

Wilson, G.I., 1961. Serum protein changes in lambs and kids after exposure to the thread lungworm Dictyocaulus filaria. Jour. Parasit. 47 (4-Sec 12): 20-21.